PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/022605	14.07.2004	14.07.2003

International Patent Classification (IPC) or both national classification and IPC
C07K14/44, C12N15/30, A61K39/018, G01N33/569, C07K16/20, C12N15/10

Applicant
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Written Opinion
2 MONTH REMINDER 6/9/05
1 MONTH REMINDER 7/9/05
2 WEEK REMINDER 7/23/05
3 DAY REMINDER 8/6/05
ACTION DUE AND DATE 8/9/05 SH

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/022605

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/022605

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 74, 77, 84, 85 (completely); 78 (partially); 55-60 (IA)

because:

☒ the said international application, or the said claims Nos. 55-60 (IA) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 74,77, 84, 85 (completely); 78 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
☐ has not been furnished
☐ does not comply with the standard

the computer readable form
☐ has not been furnished
☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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PCT/US2004/022605

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-73, 75-76, 79-83 (completely); 78 (partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8, 9, 12-14, 16-20, 22, 23, 30, 33-35, 37, 39, 46, 50-73, 75-76, 78-83
	No:	Claims	1-7, 10, 11, 15, 21, 24-29, 31-32, 36, 38, 40-45, 47-49
Inventive step (IS)	Yes:	Claims	8, 9, 12-14, 16-20, 22, 23, 30, 33-35, 37, 39, 46, 52-62, 67-72
	No:	Claims	50, 51, 63-66, 73, 75-76, 78-83
Industrial applicability (IA)	Yes:	Claims	1-54, 61-73, 75-56, 78-83
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III.

Claims 55-60 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of the above claims on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV.

1. This Authority considers that there are 7 separate inventions covered by the claims indicated as follows:

Invention (1): Claims 1-11, 24-28, 39-72 (all partially); 12,17,18,19, 29 and 34 (completely) Isolated polypeptide Tp1 comprising a sequence represented by SEQ ID NO: 1 and the antigenic fragments SEQ ID Nos: 6, 7 and 9, pharmaceutical or immunogenic composition or vaccine comprising said polypeptide, isolated polynucleotide comprising SEQ ID NO. 18 or 23 pharmaceutical composition comprising said polynucleotide, vector comprising said polynucleotide, host cell comprising said vector, method of producing a polypeptide, comprising culturing said host cell, antibody specific for the polypeptide having SEQ ID NO: 1, 6, 7 or 9, kit comprising said antibody, method for protecting an animal against infection by *T. parva,* comprising administration of said polypeptide or of said host cell, method of detecting protozoan infection, method for preparing a polyclonal or monoclonal antibody

against said polypeptide, method for identifying *T. parva* in a sample.

Invention (2): Claims 1-11, 24-28, 39-72 (all partially); 13, 20, 30, 35 (completely) same as (1), but polypeptide Tp4 comprising a sequence represented by SEQ ID NO: 2 and antigenic fragment SEQ ID NO: 14, polynucleotide comprising SEQ ID NO: 19 and 28.

Invention (3): Claims 1-11, 24-28, 39-72 (all partially); 14, 21, 31, 36, 37 (completely) as (1), but polypeptide Tp5 comprising SEQ ID NO: 3 and the antigenic fragment SEQ ID NO: 15 and polynucleotides comprising SEQ ID NO. 20, 29 and 30.

Invention (4): Claims 1-11, 24-28 and 39-72 (all partially), 15, 22, 32 (completely) as (1); but polypeptide Tp7 comprising SEQ ID NO: 4, the antigenic fragment SEQ ID NO: 16 and polynucleotide comprising SEQ ID NO: 21.

Invention (5): Claims 1-11, 24-28, 39-72 (all partially); 16, 23, 33, 38 (completely) as (1); but polypeptide Tp8 comprising SEQ ID NO: 5, the antigenic fragment SEQ ID NO: 17 and polynucleotide comprising SEQ ID NO. 22 and 31.

Invention (6): Claims 73, 75-76, 79-83 (completely); 78 (partially)
Method for the identification of parasite antigens that are targets of cytotoxic T cells.

Invention (7): Claims 74, 77, 84, 85 (completely), 78 (partially)
Method for a three-way matrix resolution for identification of a single cDNA clone from a pool of cDNAs.

2. The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

2.1 Lack of unity a priori:

Inventions 1-5: Claims 1-72 are directed to the isolation of *Theileria parva* antigens and to their use in diagnosis, therapy and prevention of disease.

Invention 6: Claim 73 is directed to a method for the identification of any parasite antigens. None of the claims dependent thereon is restricted to the *Theileria* antigens of the first invention. The claimed method is not specifically designed for the identification of the *Theileria* antigens of present application. The subject-matter of Claims 73, 75-76, 79-83 and of Claim 78 (partially) is therefore not linked by a special technical feature with the subject-matter of any of Claims 1-72 and thus constitutes an independent invention.

Invention 7: Claim 74 is directed to a method for a three-way matrix resolution for identification of a single cDNA clone from a pool of cDNAs. There exists no technical relationship at all between Claim 74 and the claims relating to *Theileria* antigens. Also no technical relationship is apparent with the subject-matter of Claim 73. The subject-matter of Claims 74, 77, 84-85 and 78 (partially) is therefore not linked with that of Claims 1-72 and Claim 73 by a common inventive concept and thus represents a further independent invention.

It is concluded that the three groups of inventions are not linked by a common inventive concept. No special technical feature can be distinguished which is present in the three groups of claims which would make a contribution over the prior art.

2.2. Lack of unity a posteriori:

Inventions 1-5 are directed to 5 antigens of *Theileria parva* (Tp1, Tp4, Tp5, Tp7 and Tp8) and antigenic fragments thereof, defined by reference to SEQ ID NOs. 1-7, 9 and 14-17. The 5 antigens show no significant structural relationship with each other. The only single general concept which can be identified as linking the different polypeptides claimed in Claim 1 is that they are derived from *Theileria parva* and

capable of inducing immunoprotection against *T. parva* by inducing a CTL response in cattle. However, this general concept is not novel, because *Theileria parva* antigens have already been disclosed in the prior art, see e.g. Gerhards et al., Mol. Biochem. Parasit. Vol. 68, pp. 235-245, 1994, (cited as D2) which discloses the sequence of a 90 kD heat-shock protein of *Theileria parva*. The amino acid sequence of this protein is 100% identical with that shown in SEQ ID NO: 4 of present application. US 5273744 discloses the use of the 67 kD antigen from *Theileria parva* for inducing immunoprotection.
In view of the prior art, the different sequences referred to in claim 1 do not share a significant technical feature, which makes a contribution over the prior art. Thus, each of the different proteins in present Claim 1 provides an independent solution to the problem of providing further antigens from *Theileria parva*.

Since an International Search Report has been established on inventions 1-6, this opinion is limited to the subject-matter searched.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to:

D1: DATABASE EMBL [Online] 2 January 2003 (2003-01-02), "EST629769 TpMugugaSh01 Theileria parva cDNA clone TPFAU35, mRNA sequence." XP002310756 retrieved from EBI accession no. EM_EST:BQ546142 Database accession no. BQ546142

D2: GERHARDS JOACHIM ET AL: "Sequence and expression of a 90-kilodalton heat-shock protein family member of Theileria parva" MOLECULAR AND BIOCHEMICAL PARASITOLOGY, vol. 68, no. 2, 1994, pages 235-246, XP002310752 ISSN: 0166-6851

D3: DATABASE EMBL [Online] 2 January 2003 (2003-01-02), "EST630891 TpMugugaSh01 Theileria parva cDNA clone TPFDB90, mRNA sequence."

XP002323333 retrieved from EBI accession no. EM_EST:BQ547264 Database accession no. BQ547264

D4: DATABASE EMBL [Online] 2 January 2003 (2003-01-02), "EST630890 TpMugugaSh01 Theileria parva cDNA clone TPFDB90, mRNA sequence." XP002323335 retrieved from EBI accession no. EM_EST:BQ547263 Database accession no. BQ547263

D5: DATABASE EMBL [Online] 2 January 2003 (2003-01-02), "EST630359 TpMugugaSh01 Theileria parva cDNA clone TPFAY47, mRNA sequence." XP002323336 retrieved from EBI accession no. EM_EST:BQ546732 Database accession no. BQ546732

D6: DATABASE EMBL [Online] 2 January 2003 (2003-01-02), "EST628661 TpMugugaSh01 Theileria parva cDNA clone TPFAM54, mRNA sequence." XP002323337 retrieved from EBI accession no. EM_EST:BQ545034 Database accession no. BQ545034

D7: GARDNER M J ET AL: "GENOME SEQUENCE OF THE HUMAN MALARIA PARASITE PLASMODIUM FALCIPARUM" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 419, 2002, pages 498-511, XP001156336 ISSN: 0028-0836

D8: BALLINGALL K T ET AL: "A highly sensitive, non-radioactive assay for T cell activation in cattle: applications in screening for antigens recognised by CD4<+> and CD8<+> T cells" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 239, no. 1-2, May 2000 (2000-05), pages 85-93, XP004204319 ISSN: 0022-1759

D9: MORRISON W IVAN ET AL: "Theileriosis: Progress towards vaccine development through understanding immune responses to the parasite" VETERINARY PARASITOLOGY, vol. 57, no. 1-3, 1995, pages 177-187, XP002310754 ISSN: 0304-4017

D10 MCKEEVER DECLAN J ET AL: "Novel vaccines against Theileria parva: Prospects for sustainability" INTERNATIONAL JOURNAL FOR PARASITOLOGY, vol. 28, no. 5, May 1998 (1998-05), pages 693-706, XP002310753 ISSN: 0020-7519

2. Novelty and inventive step (Art. 33(2) and (3) PCT):

2.1 D1 discloses an EST which is a partial sequence of SEQ ID NO. 18. Said sequence would hybridize under stringent conditions with the sequence represented by SEQ ID NO: 18. D1 is therefore novelty-destroying for the subject-matter of Claims 24-27, and 29.
Claims directed to the full-length polynucleotide encoding Tp1 and the Tp1 protein as defined by SEQ ID NO. 1 and fragments thereof as defined by SEQ ID Nos. 6, 7 and 9 are considered novel in view of the cited documents.

2.2 D2 discloses the amino acid sequence and the nucleotide sequence (see Database accession number TPHSP90) of the 90kD heat shock protein of T. parva. The sequence is identical to SEQ ID NO: 4 (Tp7) of present application.
It was shown that the protein of D2 is upregulated in the schizont stage.
D2 is considered novelty-destroying for the subject-matter of Claims 1-7, 10, 11, 15, 24-28, 32, 40-45 and 47-49.

The subject-matter of Claims 50-51 and 63-66 is not considered to involve the required inventive step in view of D2 because the preparation of monoclonal antibodies against a known protein is a well known routine method which the skilled person would apply without the exercise of inventive skill.

2.3 D3, D4, D5 and D6 disclose ESTs of Theileria parva which comprise SEQ ID Nos: 20, 29, 31 and a part of SEQ ID NO: 19 of present application. In their present drafting, Claims 24-27, 31, 36 and 38 lack novelty in view of said documents.

2.4 D7 discloses the genome sequence of Plasmodium falciparum. The translation initiation factor el comprises SEQ ID NO. 15 of present application. D7 is therefore novelty destroying for Claims 1, 3 and 21.

2.5 Closest prior art for assessing inventive step of Claims 1-72 are either D9 or D10. Both documents concern theoretical approaches to the development of novel

vaccines against *Theileria parva*. The importance of schizont-derived subunit vaccines is disclosed. It is proposed to identify antigens which induce CD8+ CTL responses. However, no such antigens, leave alone the antigens defined by their respective amino acid sequences (SEQ ID NO: 1-7, 9, 14-17), have been identified as potential CTL-inducing subunit vaccines prior to present invention. The problem underlying the present application is the provision of *Theileria parva* antigens which induce a CTL response. The solution, namely the identification of the specific Theileria parva antigens of present application (Tp1, Tp4, Tp5, Tp7 and Tp8) is considered to involve an inventive step. In the absence of any disclosure concerning the nature of such an antigen, it would not have been obvious for the skilled person to identify the specific antigens defined by their SEQ ID NOs. Therefore, claims directed to the specific antigenic polypeptides (insofar as they are novel), to immunogenic compositions comprising the polypeptides comprising SEQ ID NO: 1-5 (and the specific antigenic fragments thereof as represented by SEQ ID Nos: 6, 7, 9 and 14-17), to diagnostic, therapeutic or preventive methods employing said polypeptides, polynucleotides or antibodies, are considered to involve the required inventive step.

2.6 Claim 73 is directed to a method for the identification of parasite antigens. In D8 an assay for detecting bovine cytokines released from activated T cells (parasite antigen-specific CD4+ T cell lines) is described. Since it was shown that fractions of the intracellular schizont stage of T. parva can activate a parasite specific CD4+ T cell clone, this assay is suggested to be a useful tool for identifying protective Theileria antigens. It is stated in D8, p. 90, right col, last §, that an elegant approach for identifying tumour antigens by transfection of COS-7 cells with tumour-derived cDNA expression constructs, subsequent screening with CTL clones derived from immune patients and using TNF-sensitive WEHI indicator cells as a detection system has turned out as not being sufficiently sensitive in cattle, it is proposed to use the assay disclosed in D8 as an alternative to the WEHI system. Since it was shown that an alloreactive CTL clone releases class II MHC inducing factors in response to allo-recognition of transiently transfected COS-7 cells, it would be obvious to follow the suggestion in D8 and to arrive at the method of Claim 73 without the exercise of inventive skill.

In view of D8 the subject-matter of Claims 73, 75-76 and 78- 83 therefore is considered to lack the required inventive step (Art. 33(3) PCT).

Re item VIII

1. Claim 24 does not comply with the requirements of Art. 6 PCT, because the scope of said claim is unclear. The claim relates to polynucleotides which are defined in terms of a (short) nucleotide sequence **comprised** therein. It is not required that the sequences of Claim 24 (c) must hybridize under highly stringent conditions to that part of the polynucleotide defined by the specific sequence. The claim therefore covers sequences which are totally unrelated to the specific nucleotide sequences. The same objection applies to Claim 27.

2. Several claims appear to be redundant (see e.g. Claims 2, 3, 12-23: the subject-matter of said claims is already covered by Claim 1; Claims 25-38: already covered by Claim 24). The claims thus lack conciseness (Art. 6 PCT).

3. Claims directed to vaccines comprising the claimed polypeptides and to methods for protecting an animal against infection by T. parva are not supported by the description and are purely speculative (Art. 5 and 6 PCT). It has not been shown that the polypeptides are indeed immunoprotective.